

**Injection volume:** 20

**Detector:** UV 200

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**CHROMATOGRAM**

**Retention time:** 1.16

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**OTHER SUBSTANCES**

**Simultaneous:** diphenhydramine, granisetron (UV 300)

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**KEY WORDS**

stability-indicating; injections; saline

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**REFERENCE**

Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

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**SAMPLE**

**Matrix:** urine

**Sample preparation:** Dilute urine 1:3 to 1:39, inject a 50 µL aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 4 5 µm Hypersil ODS

**Mobile phase:** MeOH:250 mM pH 7.4 phosphate buffer 5:95 containing 5 mM tetrabutylammonium phosphate

**Flow rate:** 1

**Injection volume:** 50

**Detector:** UV 412 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and the mixture flowed through a 200 × 4 column packed with 100–120 mesh glass beads (dichlorodimethylsilane treated) to the detector. (Prepare reagent by diluting 0.2% 5,5'-dithiobis(2-nitrobenzoic acid) in 250 mM pH 7.4 phosphate buffer containing 10% tripotassium citrate 1:10 with water.)

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**CHROMATOGRAM**

**Retention time:** 7.5

**Limit of detection:** 75 ng

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**KEY WORDS**

post-column reaction; comparison with electrochemical detection without derivatization

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**REFERENCE**

Sidau,B.; Shaw,I.C. Determination of sodium 2-mercaptoethanesulphonate by high-performance liquid chromatography using post-column reaction colorimetry or electrochemical detection, *J.Chromatogr.*, **1984**, *311*, 234–238.

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# Mesoridazine

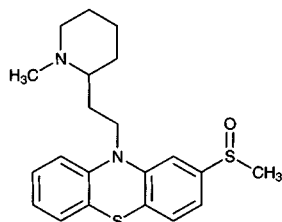
**Molecular formula:** C<sub>21</sub>H<sub>26</sub>N<sub>2</sub>OS<sub>2</sub>

**Molecular weight:** 386.58

**CAS Registry No.:** 5588-33-0, 32672-69-8 (besylate)

**Merck Index:** 5970

**Lednicer No.:** 1 389



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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 2 mL Plasma + 100 µL 1 M HCl, vortex for 30 s, add 4 mL isopropanol, mix for 5 min, centrifuge at 5000 rpm at 0° for 20 min. Remove the supernatant and adjust

the pH to 12.5 with 200  $\mu$ L 5 M NaOH, mix for 10 s, add 4 mL n-heptane, mix for 10 min, centrifuge at 2500 rpm. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 200  $\mu$ L MeCN, mix for 2 min, inject a 75-100  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 250  $\times$  3.2 5  $\mu$ m Spherisorb CN

**Mobile phase:** MeCN:15 mM pH 6.5 acetate buffer 90:10

**Flow rate:** 1.6

**Injection volume:** 75-100

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 7

**Internal standard:** mesoridazine

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**OTHER SUBSTANCES**

**Extracted:** chlorpromazine

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**KEY WORDS**

plasma; meosridazine is IS

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**REFERENCE**

Midha,K.K.; Cooper,J.K.; McGilveray,I.J.; Butterfield,A.G.; Hubbard,J.W. High-performance liquid chromatographic assay for nanogram determination of chlorpromazine and its comparison with a radioimmunoassay, *J.Pharm.Sci.*, **1981**, 70, 1043-1046.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 10 mL Plasma or whole blood + 1 mL 1 M NaOH, extract twice with 10 mL hexane for 30 min. Remove the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 1 mL 100 mM HCl, add 5 mL chloroform, vortex for 1 min, centrifuge. Remove a 4.5 mL aliquot of the organic layer and evaporate it to dryness, reconstitute the residue in 100  $\mu$ L mobile phase, inject a 50  $\mu$ L aliquot. (It is implied, but not explicitly stated in the paper, that this extraction procedure works for this compound.)

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**HPLC VARIABLES**

**Column:** 10  $\mu$ m Micropak CN (Varian)

**Mobile phase:** MeCN:20 mM ammonium acetate 90:10

**Flow rate:** 2.5

**Injection volume:** 50

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 23.9

**Limit of detection:** 10 ng/mL

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**OTHER SUBSTANCES**

**Simultaneous:** acetophenazine, amitriptyline, benztropine, butaperazine, carphenazine, chlorpromazine, fluphenazine, haloperidol, imipramine, nortriptyline, orphenadrine, piperacetazine, promazine, promethazine, thioridazine, thiothixene, trifluoperazine, triflupromazine, trihexyphenidyl, trimeprazine

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**KEY WORDS**

plasma; whole blood

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**REFERENCE**

Curry,S.H.; Brown,E.A.; Hu,O.Y.-P.; Perrin,J.H. Liquid chromatographic assay of phenothiazine, thioxanthene and butyrophenone neuroleptics and antihistamines in blood and plasma with conventional and radial compression columns and UV and electrochemical detection, *J.Chromatogr.*, **1982**, 231, 361-376.

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**SAMPLE**

**Matrix:** blood, tissue

**Sample preparation:** Blood or serum. 1 mL Blood or serum + 1 µg cianopramine + 1 mL water, vortex, add 1 mL 200 mM sodium carbonate, vortex, add 6 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 100 µL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 µL aliquot of the aqueous layer. Liver homogenate. 0.5 mL Liver homogenate + 10 µg cianopramine + 500 µL 2% sodium tetraborate + 8 mL hexane:1-butanol 95:5, gently agitate for 30 min, centrifuge at 2500 g for 5 min. Remove the organic layer and add it to 400 µL 0.2% phosphoric acid, agitate gently for 30 min, centrifuge for 5 min. Remove the organic layer and inject a 30 µL aliquot of the aqueous layer.

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**HPLC VARIABLES**

**Guard column:** 15 × 3.2 7 µm RP-18 Newguard (Applied Biosystems)

**Column:** 100 × 4.6 5 µm Brownlee Spheri-5 RP-18

**Mobile phase:** MeCN:100 mM NaH<sub>2</sub>PO<sub>4</sub>:diethylamine 40:57.5:2.5

**Flow rate:** 2

**Injection volume:** 30

**Detector:** UV 220

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**CHROMATOGRAM**

**Retention time:** 12.46

**Internal standard:** cianopramine (8.93)

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**OTHER SUBSTANCES**

**Simultaneous:** amitriptyline, amoxapine, benztropine, brompheniramine, chlorpheniramine, chlorpromazine, clomipramine, cyproheptadine, desipramine, diphenhydramine, dothiepin, doxepin, fluoxetine, haloperidol, imipramine, loxapine, maprotiline, meperidine, methadone, metoclopramide, mianserin, moclobemide, nomifensine, nordoxepin, norfluoxetine, norpropoxyphene, nortriaden, nortriptyline, pentobarbital, pheniramine, promethazine, propranolol, protriptyline, quinidine, quinine, sulforidazine, thioridazine, thiothixene, tranlycypromine, trazodone, trihexyphenidyl, trimipramine, triprolidine

**Noninterfering:** dextromethorphan, norphetidine, phenoxybenzamine, prochlorperazine, tri-fluoperazine

**Interfering:** propoxyphene

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**KEY WORDS**

serum; whole blood; liver

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**REFERENCE**

McIntyre, I.M.; King, C.V.; Skafidis, S.; Drummer, O.H. Dual ultraviolet wavelength high-performance liquid chromatographic method for the forensic or clinical analysis of seventeen antidepressants and some selected metabolites, *J.Chromatogr.*, **1993**, 621, 215–223.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Tablets. Powder tablets, weigh out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, filter (0.45 µm) (discard first 10 mL of filtrate), inject a 20 µL aliquot of the filtrate. Syrups, elixirs, injectables. Measure out amount equivalent to about 10 mg, add 75 mL mobile phase, sonicate for 20 min, dilute to 100 mL with mobile phase, mix, inject a 20 µL aliquot.

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**HPLC VARIABLES**

**Column:** 300 × 3.9 10 µm µBondapak CN

**Mobile phase:** MeOH:3 mM ammonium acetate 90:10

**Flow rate:** 1.3

**Injection volume:** 20

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 5.2

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**OTHER SUBSTANCES**

**Also analyzed:** chlorpheniramine, cyclizine, doxylamine, pentazocine, promethazine, protriptyline, pyrilamine, pyrimethamine, tripeleminamine

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**KEY WORDS**

tablets; syrups; elixirs; injections

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**REFERENCE**

Walker, S.T. Liquid chromatographic determination of organic nitrogenous bases in dosage forms: a progress report, *J. Assoc. Off. Anal. Chem.*, **1985**, *68*, 539–542.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

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**HPLC VARIABLES**

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

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**CHROMATOGRAM**

**Retention time:** 5.3

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**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocetamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazepine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipranone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenazepam, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenylglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxylbenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenylamine, theophylline, thiethylperazine, thiopropazate, thiopropazine, thioridazine, thiothixene, thonzylamine, timolol, tocanide, tolpropamine, tolycaine, tranlycypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

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**REFERENCE**

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50 µg/mL, inject a 10 µL aliquot.

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**HPLC VARIABLES**

**Column:** 300 × 3.9 10 µm µBondapak C18

**Mobile phase:** MeOH:acetic acid:triethylamine:water 70:1.5:0.5:28

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 5

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**OTHER SUBSTANCES**

**Simultaneous:** promethazine, acetophenazine, chlorpromazine, thioridazine, prochlorperazine, butaperazine, thiethylperazine

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**REFERENCE**

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Ultrasphere cyano

**Mobile phase:** MeCN:10 mM pH 2.5 KH<sub>2</sub>PO<sub>4</sub> 60:40

**Flow rate:** 2.5

**Injection volume:** 20-40

**Detector:** E, Environmental Science Associates Coulochem Model 5100A, Model 5100 guard-cell +0.85 V (between pump and injector), Model 5010 analytical cell +0.8 V, preanalytical cell +0.3 V

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**CHROMATOGRAM**

**Retention time:** 4.0

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**OTHER SUBSTANCES**

**Simultaneous:** amitriptyline, chlorpromazine, desipramine, doxepin, fluphenazine, haloperidol, imipramine, loxapine, nortriptyline, perphenazine, pheniramine, phenylephrine, prochlorperazine, promazine, promethazine, thioridazine, thiothixene, triflupromazine, trimeprazine, tripeleennamine

**Noninterfering:** diazepam, diphenhydramine, ethopropazine, fluoxetine, nordiazepam, oxazepam, phenylpropanolamine, pseudoephedrine, trifluoperazine

**Interfering:** amoxapine, reduced haloperidol, desmethyldoxepin, trazodone

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**REFERENCE**

Hariharan,M.; VanNoord,T.; Kindt,E.K.; Tandon,R. A simple, sensitive liquid chromatographic assay of cis-thiothixene in plasma with coulometric detection, *Ther.Drug Monit.*, **1991**, *13*, 79–85.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Guard column:** 30 × 2.1 Spheri-5 RP-8

**Column:** 220 × 2.1 Spheri-5 RP-8

**Mobile phase:** Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.

**Column temperature:** 50

**Flow rate:** 0.5

**Detector:** UV 200

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## CHROMATOGRAM

**Retention time:** 12.5

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## OTHER SUBSTANCES

**Simultaneous:** promazine, thiothixene, chlorpromazine, trifluoperazine, thioridazine

**Also analyzed:** amitriptyline, amphetamine, chlordiazepoxide, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, diethylpropion, doxepin, ephedrine, fenfluramine, flurazepam, imipramine, methamphetamine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, phentermine, phenylpropanolamine, prazepam

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## REFERENCE

*Rainin Catalog, C1-94, 1994, p. 7.24.*

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Column:** 250 × 4.6 Zorbax RX

**Mobile phase:** Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

**Column temperature:** 30

**Flow rate:** 2

**Detector:** UV 210

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## OTHER SUBSTANCES

**Also analyzed:** acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepam, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, ethorphine, eugenol, famotidine, fenbendazole, fenecamfamine, fenpropofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaicol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarboxtyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephénytoin, mephesin, mephobarbital, mepivacaine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyl dopa, methyl dopamine, methylphenidate, methylprednisolone, methyltestosterone, methylprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nyldrin, oxazepam, oxycodone, oxymorphone,

oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxazole, sulfafethoxazole, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfasoxazole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranlycypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripeleminamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

## REFERENCE

Hill, D.W.; Kind, A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J. Anal. Toxicol.*, **1994**, *18*, 233–242.

## SAMPLE

**Matrix:** solutions

## HPLC VARIABLES

**Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4.5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

## CHROMATOGRAM

**Retention time:** 10.12 (A), 5.02 (B)

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordi-azepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazin-  
dol, mefenamic acid, meperidine, mephénytoin, mepivacaine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methylldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclo-  
bemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemo-  
line, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimo-  
zide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, pro-  
piomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, so-  
talol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetra-

caine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocinide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimipramine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

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**KEY WORDS**

details of plasma extraction

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**REFERENCE**

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, 692, 103–119.

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**SAMPLE**

**Matrix:** urine

**Sample preparation:** Perform all procedures in subdued light. 10 mL Urine + 10  $\mu$ L 100  $\mu$ g/mL IS, lyophilize, extract residue with 3 mL MeOH by shaking for 15 min, repeat extraction twice, combine extracts and evaporate them to dryness under vacuum below 45°, dissolve residue in 2 mL 300 mM pH 7.2 phosphate buffer, extract three times with 3 mL dichloromethane, wash the combined organic layers twice with 2 mL phosphate buffer, twice with 2 mL water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute with 100  $\mu$ L MeCN, inject a 10  $\mu$ L aliquot.

---

**HPLC VARIABLES**

**Column:** 150  $\times$  4.6 3  $\mu$ m Spherisorb cyano

**Mobile phase:** MeCN:50 mM ammonium acetate:diethylamine 92:8:0.05

**Flow rate:** 1.1

**Injection volume:** 10

**Detector:** UV 254

---

**CHROMATOGRAM**

**Retention time:** 10.9

**Internal standard:** prochlorperazine ring sulfoxide (14)

**Limit of quantitation:** 20 ng/mL

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**OTHER SUBSTANCES**

**Simultaneous:** metabolites

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**KEY WORDS**

human; rat; dog

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**REFERENCE**

Lin,G.; Hawes,E.M.; McKay,G.; Korchinski,E.D.; Midha,K.K. Metabolism of piperidine-type phenothiazine antipsychotic agents. IV. Thioridazine in dog, man and rat, *Xenobiotica*, **1993**, 23, 1059–1074.

---

**SAMPLE**

**Matrix:** urine

**Sample preparation:** Perform all procedures in subdued light. 10 mL Urine + 10  $\mu$ L 100  $\mu$ g/mL IS, lyophilize, extract residue with 3 mL MeOH by shaking for 15 min, repeat extraction twice, combine extracts and evaporate them to dryness under vacuum below 45°, dissolve residue in 2 mL 300 mM pH 7.2 phosphate buffer, extract three times with 3 mL dichloromethane, wash the combined organic layers twice with 2 mL phosphate buffer, twice with 2 mL water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute with 100  $\mu$ L MeCN, inject a 10  $\mu$ L aliquot.

---

**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu$ m Serva Si 100 polyol (Terochem Laboratories)

**Mobile phase:** MeCN:47 mM pH 7.8 ammonium acetate 83:17

**Flow rate:** 1

**Injection volume:** 10

**Detector:** MS plasm spray, VG 70SQ, discharge 320 V, ion source 250°, plasm spray probe tip 200–300



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**CHROMATOGRAM****Retention time:** 5.72

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**OTHER SUBSTANCES****Simultaneous:** metabolites, sulforidazine, thioridazine

---

**KEY WORDS**human; rat; dog

---

**REFERENCE**

Lin,G.; Hawes,E.M.; McKay,G.; Korchinski,E.D.; Midha,K.K. Metabolism of piperidine-type phenothiazine antipsychotic agents. IV. Thioridazine in dog, man and rat, *Xenobiotica*, **1993**, 23, 1059–1074.

---

**SAMPLE****Matrix:** urine

**Sample preparation:** Human urine. 10 mL Urine + 1 mL 10 µg/mL IS, lyophilize, add 3 mL MeOH, shake for 15 min, repeat extraction twice more. Combine the extracts and evaporate them to dryness under reduced pressure below 45°, reconstitute the residue in 2 mL 300 mM pH 7.2 phosphate buffer, extract three times with 3 mL dichloromethane. Combine the organic extracts and wash them twice with 2 mL phosphate buffer, wash twice with 2 mL water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute in 100 µL MeCN, inject a 10 µL aliquot. Dog, rat urine. 1 mL Urine + 50 µL 100 µg/mL IS, lyophilize, add 3 mL MeOH, shake for 15 min, repeat extraction twice more. Combine the extracts and evaporate them to dryness under reduced pressure below 45°, reconstitute the residue in 2 mL 300 mM pH 7.2 phosphate buffer, extract three times with 3 mL dichloromethane. Combine the organic extracts and wash them twice with 2 mL phosphate buffer, wash twice with 2 mL water, dry over anhydrous sodium sulfate, evaporate to dryness, reconstitute in 100 µL MeCN, inject a 10 µL aliquot.

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**HPLC VARIABLES****Column:** 150 × 4.6 3 µm Spherisorb cyano**Mobile phase:** MeCN:50 mM ammonium acetate:diethylamine 92:8:0.05**Flow rate:** 1**Injection volume:** 10**Detector:** UV 254

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**CHROMATOGRAM****Retention time:** 11.3**Internal standard:** prochlorperazine ring sulfoxide**Limit of quantitation:** 10 ng/mL

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**OTHER SUBSTANCES****Extracted:** metabolites

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**KEY WORDS**dog; rat; human; protect from light

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**REFERENCE**

Lin,G.; Hawes,E.M.; McKay,G.; Korchinski,E.D.; Midha,K.K. The metabolism of piperidine-type phenothiazine antipsychotic agents. III. Mesoridazine in dog, human and rat, *Xenobiotica*, **1993**, 23, 37–52.

# Mestranol

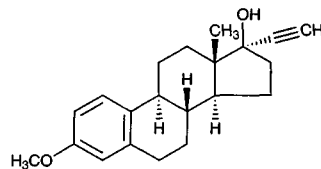
**Molecular formula:**  $C_{21}H_{26}O_2$

**Molecular weight:** 310.44

**CAS Registry No.:** 72-33-3

**Merck Index:** 5976

**Lednicer No.:** 1 162



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## SAMPLE

**Matrix:** formulations

**Sample preparation:** Powder tablets (60 mesh), weigh out amount equivalent to one tablet, add 2 mL 50  $\mu$ g/mL BHT in MeCN:water 80:20, shake 30 min, centrifuge

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## HPLC VARIABLES

**Column:** 250  $\times$  3.2 Altex RP-2 express series

**Mobile phase:** MeCN:water 38:62

**Flow rate:** 1.75

**Injection volume:** 20

**Detector:** UV 210 or 280

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## CHROMATOGRAM

**Retention time:**  $k'$  8.08

**Internal standard:** BHT (butylated hydroxytoluene) ( $k'$  16.54)

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## OTHER SUBSTANCES

**Simultaneous:** ethynodiol diacetate, ethinyl estradiol, degradation products

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## KEY WORDS

tablets

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## REFERENCE

Carignan, G.; Lodge, B.A.; Skakum, W. Quantitative analysis of ethynodiol diacetate and ethinyl estradiol/mestranol in oral contraceptive tablets by high-performance liquid chromatography, *J.Pharm.Sci.*, **1982**, 71, 264-266.

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## SAMPLE

**Matrix:** formulations

**Sample preparation:** Dissolve 6 tablets in 600 mL dissolution medium (water:isopropanol 97:3), remove 5 mL samples, centrifuge at 1500 rpm for 10 min, inject a 50-200  $\mu$ L aliquot.

---

## HPLC VARIABLES

**Column:** 300  $\times$  3.9 10  $\mu$ m Bondapak C18

**Mobile phase:** MeCN:water 55:45

**Flow rate:** 1

**Injection volume:** 50-200

**Detector:** UV 200

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## OTHER SUBSTANCES

**Simultaneous:** norethindrone

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## KEY WORDS

tablets; modified USP method

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## REFERENCE

Nguyen, H.T.; Shiu, G.K.; Worsley, W.N.; Skelly, J.P. Dissolution testing of norethindrone:ethinyl estradiol, norethindrone:mestranol, and norethindrone acetate:ethinyl estradiol combination tablets, *J.Pharm.Sci.*, **1990**, 79, 163-167.

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**SAMPLE**

**Matrix:** microsomal incubations

**Sample preparation:** 500  $\mu$ L Microsomal incubation + 100  $\mu$ L MeCN + IS, centrifuge at 16000 g for 5 min. Inject an aliquot of the supernatant.

---

**HPLC VARIABLES**

**Column:** 300  $\times$  3.9  $\mu$ Bondapack C18

**Mobile phase:** MeCN:50 mM pH 6 potassium phosphate buffer 47:53

**Flow rate:** 1.5

**Detector:** UV 208

---

**CHROMATOGRAM**

**Retention time:** 23-24

**Internal standard:** estradiol-3-acetate (13-14)

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**OTHER SUBSTANCES**

**Simultaneous:** metabolites

**Noninterfering:** fluconazole, itraconazole, miconazole,  $\alpha$ -naphthoflavone, quinidine, sulfaphenazole, toleandomycin

**Interfering:** ketoconazole

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**KEY WORDS**

liver

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**REFERENCE**

Schmider,J.; Greenblatt,D.J.; von Moltke,L.L.; Karsov,D.; Vena,R.; Friedman,H.L.; Shader,R.I. Biotransformation of mestranol to ethinyl estradiol in vitro: The role of cytochrome P-450 2C9 and metabolic inhibitors, *J.Clin.Pharmacol.*, **1997**, 37, 193-200.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Dissolve in MeOH:water 1:1 at a concentration of 50  $\mu$ g/mL, inject a 10  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** 300  $\times$  3.9 10  $\mu$ m  $\mu$ Bondapak C18

**Mobile phase:** MeOH:acetic acid:triethylamine:water 80:1.5:0.5:18

**Flow rate:** 1.5

**Injection volume:** 10

**Detector:** UV

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**CHROMATOGRAM**

**Retention time:** k' 1.51

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**REFERENCE**

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, 370, 403-418.

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**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Extract 15 mL water with dichloromethane, evaporate organic layer, take up residue in 3 mL mobile phase, inject 50  $\mu$ L aliquot.

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**HPLC VARIABLES**

**Column:** reverse phase

**Mobile phase:** MeOH:water 82:18

**Injection volume:** 50

**Detector:** F ex 200 em 300

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**CHROMATOGRAM**

**Internal standard:** mestranol

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**OTHER SUBSTANCES**

**Simultaneous:** ethinylestradiol

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**KEY WORDS**

mestranol is IS

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**REFERENCE**

de Leede, L.G.J.; Govers, C.P.M.; de Nijs, H. A multi-compartment vaginal ring system for independently adjustable release of contraceptive steroids, *Contraception*, **1986**, 34, 589–602.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm LiChrosorb Si 60

**Mobile phase:** Hexane:dioxane:isopropanol 95:3:2

**Flow rate:** 1

**Detector:** UV 254

---

**CHROMATOGRAM**

**Retention time:** 6

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**OTHER SUBSTANCES**

**Simultaneous:** estrone, ethinyl estradiol, norethindrone, norethindrone acetate, norgestrel

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**KEY WORDS**

normal phase

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**REFERENCE**

Gazdag, M.; Szepesi, G.; Fábíán-Varga, K. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. II. Optimization for selectivity in normal-phase systems, *J.Chromatogr.*, **1988**, 454, 95–107.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 10 µm Nucleosil C18

**Mobile phase:** MeCN:THF:water 12.9:22.4:64.7

**Flow rate:** 1

**Detector:** UV 240

---

**CHROMATOGRAM**

**Retention time:** 37

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**OTHER SUBSTANCES**

**Simultaneous:** estrone, ethinyl estradiol, norethindrone, norethindrone acetate, norgestrel

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**REFERENCE**

Gazdag, M.; Szepesi, G.; Szeleczi, E. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis. I. Optimization for selectivity in reversed-phase chromatography, *J.Chromatogr.*, **1988**, 454, 83–94.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Guard column:** 12 × 6 Zorbax Reliance

**Column:** 150 × 4.6 Zorbax ODS

**Mobile phase:** MeCN:pH 3.8 acetate buffer 70:30 containing 0.1 mM tetrabutylammonium phosphate

**Flow rate:** 0.9

**Injection volume:** 100

**Detector:** UV 280

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#### CHROMATOGRAM

**Retention time:** 4

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#### REFERENCE

Patel, J.U.; Prankerd, R.J.; Sloan, K.B. A prodrug approach to increasing the oral potency of a phenolic drug. 1. Synthesis, characterization, and stability of an *O*-(imidomethyl) derivative of 17 $\beta$ -estradiol, *J.Pharm.Sci.*, **1994**, 83, 1477–1481.

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#### SAMPLE

**Matrix:** solutions

**Sample preparation:** Inject a 20  $\mu$ L aliquot of a solution in MeOH:water 50:50.

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#### HPLC VARIABLES

**Column:** 250 × 4 7  $\mu$ m LichroCART RP-8 (Merck)

**Mobile phase:** MeCN:MeOH:water 32:37:31

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 230

---

#### CHROMATOGRAM

**Retention time:** 11

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#### OTHER SUBSTANCES

**Simultaneous:** fluoxymesterone, medrogestone, norethindrone, progesterone, testosterone propionate

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#### REFERENCE

Gau, Y.S.; Sun, S.W.; Chem, R.R.-L. Optimization of high-performance liquid chromatographic separation for progestogenic, estrogenic, and androgenic steroids using factorial design, *J.Liq.Chromatogr.*, **1995**, 18, 2373–2382.

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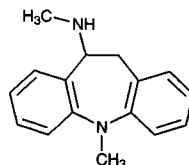
# Metopramine

**Molecular formula:** C<sub>16</sub>H<sub>18</sub>N<sub>2</sub>

**Molecular weight:** 238.33

**CAS Registry No.:** 21730-16-5

**Merck Index:** 5991



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#### SAMPLE

**Matrix:** blood

**Sample preparation:** 1 mL Plasma + 50  $\mu$ L 10  $\mu$ g/mL chlorohaloperidol in MeOH + 50  $\mu$ L 1 M NaOH + 6 mL distilled ether, shake mechanically for 10 min, centrifuge for 5 min. Remove the organic layer and add it to 100  $\mu$ L 100 mM HCl, shake for 10 min, centrifuge for 5 min. Remove the aqueous layer, centrifuge for 2 min, inject a 60  $\mu$ L aliquot.

---

#### HPLC VARIABLES

**Column:** 125 × 4.9 5  $\mu$ m Spherisorb C8

**Mobile phase:** MeCN:MeOH:buffer 13:35:52 (Buffer was 6.5 g/L (?) KH<sub>2</sub>PO<sub>4</sub> adjusted to pH 3 with orthophosphoric acid.)

**Column temperature:** 45

**Flow rate:** 1.2  
**Injection volume:** 60  
**Detector:** UV 254

---

#### CHROMATOGRAM

**Retention time:** 3.65  
**Internal standard:** chlorohaloperidol (6.83)  
**Limit of quantitation:** 3 ng/mL

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#### OTHER SUBSTANCES

**Extracted:** metabolites  
**Simultaneous:** amitriptyline, bromazepam, chlorpromazine, clobazam, clomipramine, desipramine, diazepam, flunitrazepam, haloperidol, imipramine, indalpine, levomepromazine, lorazepam, maprotiline, mianserin, oxazepam, prochlorperazine, triazolam  
**Noninterfering:** meprobamate

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#### KEY WORDS

plasma

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#### REFERENCE

Rouquette,C.; Hecquet,D.; Pommereau,X.; Gardere,J.J.; Brachet-Liermain,A. Metapramine overdose: report of two cases and analytical determinations, *J.Anal.Toxicol.*, **1985**, 9, 275–277.

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#### SAMPLE

**Matrix:** blood

**Sample preparation:** Evaporate 200  $\mu$ L 1  $\mu$ g/mL citalopram in MeOH into a tube, add 2 mL plasma, add 2 mL pH 10 Titrisol buffer (Merck), add 8 mL diethyl ether, shake for 15 min, centrifuge at 2800 g for 5 min. Remove the organic phase and shake it with 100  $\mu$ L 50 mM phosphoric acid for 15 min, centrifuge at 2800 g for 10 s. Remove the aqueous layer and vortex it with 2 mL diethyl ether for 10 s, centrifuge at 2800 g. Discard the organic layer and inject a 10-50  $\mu$ L aliquot of the aqueous layer.

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#### HPLC VARIABLES

**Column:** 300  $\times$  3.9 10  $\mu$ m  $\mu$ Bondapak C18  
**Mobile phase:** MeCN:25 mM  $\text{KH}_2\text{PO}_4$ :water 45:55:10  
**Flow rate:** 0.6  
**Injection volume:** 10-50  
**Detector:** UV 254

---

#### CHROMATOGRAM

**Retention time:** 8.6  
**Internal standard:** citalopram (10.8)  
**Limit of detection:** 5 ng/mL

---

#### OTHER SUBSTANCES

**Simultaneous:** metabolites  
**Noninterfering:** diazepam, amitriptyline, clobazam, levomepromazine, norclobazam, triazolam, monodesmethyltrimipramine, flunitrazepam, alimemazine, alprazolam, amineptine, caffeine, carbamazepine, desmethylflunitrazepam, diazepam, dibenzepine, estazolam, ethyl loflazepate, loprazolam, lorazepam, meprobamate, nitrazepam, nordiazepam, nortriptyline, oxazepam, viloxazine  
**Interfering:** indalpine

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#### KEY WORDS

plasma

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#### REFERENCE

Pok Phak,R.; Conquy,T.; Gouezo,F.; Viala,A.; Grimaldi,F. Determination of metapramine, imipramine, trimipramine and their major metabolites in plasma by reversed-phase column liquid chromatography, *J.Chromatogr.*, **1986**, 375, 339–347.

**SAMPLE****Matrix:** blood

**Sample preparation:** 2 mL Plasma + 200  $\mu$ L MeOH + 2 mL 1 M pH 10.0 phosphate buffer + 6 mL diethyl ether:hexane 50:50, shake for 15 min, centrifuge at 4000 g for 5 min. Remove the organic layer and add it to 2 mL 62.5 mM sulfuric acid, vortex for 5 min, centrifuge at 4000 g for 5 min. Remove the aqueous phase and add it to 1 mL 500 mM NaOH, vortex, add 6 mL hexane:diethyl ether 50:50, shake for 10 min, centrifuge at 4000 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 100 mM sodium carbonate, add 10  $\mu$ L 10 mg/mL dansyl chloride in acetone, vortex for 1 min, heat at 45° for 30 min, evaporate under a stream of nitrogen at 50°. Reconstitute the residue in 200  $\mu$ L MeCN:water 45:55, inject a 100  $\mu$ L aliquot.

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**HPLC VARIABLES****Column:** 125  $\times$  4.6 5  $\mu$ m Hypersil ODS**Mobile phase:** Gradient. MeCN:water from 45:55 to 65:35 over 10 min, maintain at 65:35 for 20 min**Column temperature:** 30**Flow rate:** 1.5**Injection volume:** 100**Detector:** F ex Fluorichrom 7.54 and 7.60 filters, em 3.71 and 4.76 filters

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**CHROMATOGRAM****Retention time:** 28**Internal standard:** metopramine**Limit of detection:** 1.5 ng/mL

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**OTHER SUBSTANCES****Extracted:** fluvoxamine

**Noninterfering:** alimemazine, alprazolam, amineptine, amitriptyline, caffeine, clobazam, clomipramine, clorazepate, cyamemazine, diazepam, demethyldiazepam, flunitrazepam, levomepromazine, loprazolam, lorazepam, meprobamate, nitrazepam, oxazepam, triazolam, viloxazine

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**KEY WORDS**

plasma; protect from light; derivatization; metopramine is IS

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**REFERENCE**

Pommery,J.; Lhermitte,M. High performance liquid chromatographic determination of fluvoxamine in human plasma, *Biomed.Chromatogr.*, **1989**, 3, 177-179.

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**SAMPLE****Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol:n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100  $\mu$ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50  $\mu$ L aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

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**HPLC VARIABLES****Column:** 300  $\times$  3.9 4  $\mu$ m NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic))  $\text{KH}_2\text{PO}_4$  adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30**Flow rate:** 0.8**Injection volume:** 50**Detector:** UV 269

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**CHROMATOGRAM****Retention time:** 5.08**Limit of detection:** <120 ng/mL

**KEY WORDS**

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfapyrazole; flumazenil; sulpride; morphine; atenolol; tolaxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; ace-nocoumarol; vandesine; mexiletine; dipyrindamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrridine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; prazepam; alimemazine; loperamide; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thiopropazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpi-pramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

**REFERENCE**

Tracqui, A.; Kintz, P.; Mangin, P. Systematic toxicological analysis using HPLC/DAD, *J. Forensic Sci.*, **1995**, *40*, 254–262.

**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Plasma. 0.5–2 mL Plasma + 10–1000 ng maprotiline in water + 2 mL pH 10 phosphate buffer + 6 mL diethyl ether:hexane 50:50, shake for 15 min, centrifuge at 3750 g for 5 min. Remove the organic phase and add it to 2 mL 250 mM sulfuric acid, shake for 10 min, centrifuge for 5 min, discard the organic layer. Adjust the pH of the aqueous phase to 9.5–10.5 with 500 mM NaOH containing 1 M  $K_2HPO_4$ , add 6 mL diethyl ether:hexane 50:50, shake for 10 min, centrifuge for 10 min. Remove the organic layer and evaporate it to dryness under vacuum and a stream of nitrogen at 45°, reconstitute the residue in 100  $\mu$ L 100 mM sodium carbonate, add 10  $\mu$ L 1% dansyl chloride in acetone, vortex for 20–30 s, heat at 45° for 30 min. Evaporate the solvent, reconstitute in 100  $\mu$ L mobile phase, inject a 10–50  $\mu$ L aliquot. Urine. 0.5–2 mL Urine + 10–1000 ng maprotiline in water + 2 mL pH 10 phosphate buffer + 6 mL diethyl ether:hexane 50:50, shake for 15 min, centrifuge at 3750 g for 5 min. Remove the organic phase and evaporate it to dryness under vacuum and a stream of nitrogen at 45°, reconstitute the residue in 100  $\mu$ L 100 mM sodium carbonate, add 10  $\mu$ L 1% dansyl chloride in acetone, vortex for 20–30 s, heat at 45° for 30 min. Evaporate the solvent, reconstitute in 100  $\mu$ L mobile phase, inject a 10–50  $\mu$ L aliquot.

**HPLC VARIABLES**

**Column:** 125  $\times$  4.5  $\mu$ m LiChrosorb RP-18

**Mobile phase:** MeCN:water 65:35

**Flow rate:** 2

**Injection volume:** 10–50



**Detector:** F ex 248 em 470

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**CHROMATOGRAM**

**Retention time:** 16.2

**Internal standard:** maprotiline (18.5)

**Limit of detection:** 1 ng/mL

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**OTHER SUBSTANCES**

**Extracted:** metabolites

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**KEY WORDS**

plasma; derivatization; pharmacokinetics

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**REFERENCE**

Sommadossi,J.P.; Lemar,M.; Necciari,J.; Sumirtapura,Y.; Cano,J.P.; Gaillot,J. High-performance liquid chromatographic method for the determination of plasma and urine metapramine after dansylation, *J.Chromatogr.*, **1982**, 228, 205–213.

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**SAMPLE**

**Matrix:** microsomal incubations

**Sample preparation:** 1 mL Microsomal incubation + 100 µL 1 M NaOH + 25 µL 4 mM desipramine in MeOH, extract twice with diethyl ether. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 µL, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 4 Lichrosorb RP18

**Mobile phase:** Gradient. A was MeCN:20 mM sulfuric acid 20:80. B was MeCN:20 mM sulfuric acid 90:10. A:B from 100:0 to 0:100 over 20 min.

**Flow rate:** 1

**Detector:** UV 275

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**CHROMATOGRAM**

**Retention time:** 10.6

**Internal standard:** desipramine (13.3)

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**OTHER SUBSTANCES**

**Extracted:** metabolites

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**KEY WORDS**

rat; liver

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**REFERENCE**

Barret,R.; Jaussaud,P.; Pautet,F.; Guyot,J.L.; Daudon,M. Metabolism of metapramine in vitro by chemical model systems and rat liver microsomes, *Arzneimittelforschung*, **1989**, 39, 1574–1576.

---

# Metaproterenol

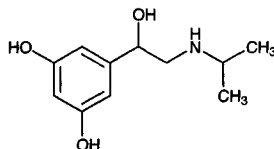
**Molecular formula:** C<sub>11</sub>H<sub>17</sub>NO<sub>3</sub>

**Molecular weight:** 211.26

**CAS Registry No.:** 586-06-1, 5874-97-5 (sulfate)

**Merck Index:** 5992

**Lednicer No.:** 1 64



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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Condition a Bond-Elut C18 SPE cartridge with MeCN and water. 1 mL Plasma + 100  $\mu$ L 2  $\mu$ g/mL terbutaline sulfate + 1 mL 20 mM pH 9.0 Na<sub>2</sub>HPO<sub>4</sub>, mix, add to the SPE cartridge, wash with 5 column volumes of water, dry the SPE cartridge for 5 min, wash with 3 mL dichloromethane:n-butanol 97:3, elute with two 1 mL portions of 0.09% HCl in MeCN, evaporate the eluate to dryness under a stream of nitrogen at 37°, dissolve the residue in 300  $\mu$ L mobile phase, inject a 200  $\mu$ L aliquot. (To hydrolyze 3-O-metaproterenol sulfate mix 1 mL plasma and 200  $\mu$ L 2  $\mu$ g/mL terbutaline sulfate, add 1 mL 6% trichloroacetic acid, vortex, centrifuge at 1000 g for 15 min. Remove the supernatant and add it to 200  $\mu$ L 2 M HCl, heat at 65° for 90 min, cool, adjust pH to 10 with 400  $\mu$ L 2 M carbonate buffer, proceed as above.)

---

#### HPLC VARIABLES

**Column:** 250  $\times$  4.9 5  $\mu$ m Spherisorb C8

**Mobile phase:** MeCN:buffer:water 4:1.5:94.5

**Flow rate:** 1.8

**Injection volume:** 200

**Detector:** F ex 200 em 300 (cut-off filter)

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#### CHROMATOGRAM

**Retention time:** 6.9

**Internal standard:** terbutaline (14.8)

**Limit of quantitation:** 0.5 ng/mL

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#### KEY WORDS

SPE; plasma; pharmacokinetics

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#### REFERENCE

Selinger,K.; Hill,H.M.; Matheou,D.; Dehelean,L. Determination of free and total metaproterenol in human plasma by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1989**, 493, 230-238.

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#### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

---

#### HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250  $\times$  4.6 5  $\mu$ m Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 200.5

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#### CHROMATOGRAM

**Retention time:** 4.15

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#### KEY WORDS

whole blood

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**REFERENCE**

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, 763, 149–163.

---

**SAMPLE**

**Matrix:** solutions

**Sample preparation:** Evaporate an aliquot of a solution in MeCN containing 625 ng drug to dryness under a stream of nitrogen at room temperature, add 200  $\mu$ L saturated sodium carbonate, add 200  $\mu$ L 4% (-)-menthyl chloroformate in MeCN, vortex for 30 s, add an excess amount of 4-hydroxy-L-proline, vortex for 30 s, centrifuge for 3 min, inject a 10–25  $\mu$ L aliquot of the upper layer.

---

**HPLC VARIABLES**

**Guard column:** 50  $\times$  4.6 Pellicular ODS (Whatman)

**Column:** 100  $\times$  4.6 5  $\mu$ m Partisil 5 ODS3

**Mobile phase:** MeOH:water 60:40

**Flow rate:** 1

**Injection volume:** 10–25

**Detector:** F ex 232 em no emission filter

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**CHROMATOGRAM**

**Retention time:** 19, 21 (enantiomers)

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**OTHER SUBSTANCES**

**Simultaneous:** sotalol

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**KEY WORDS**

derivatization; chiral

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**REFERENCE**

Mehvar, R. Stereospecific liquid chromatographic analysis of racemic adrenergic drugs utilizing precolumn derivatization with (-)-menthyl chloroformate, *J. Chromatogr.*, **1989**, 493, 402–408.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 Chirex 3020 (Phenomenex)

**Mobile phase:** Hexane:1,2-dichloroethane:EtOH/trifluoroacetic acid 55:35:10 (EtOH/trifluoroacetic acid was premixed 20:1.)

**Flow rate:** 0.7–1

**Injection volume:** 20

**Detector:** UV 278

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**KEY WORDS**

chiral;  $\alpha = 1.24$  for enantiomers

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**REFERENCE**

Cleveland, T. Pirkle-concept chiral stationary phases for the HPLC separation of pharmaceutical racemates, *J. Liq. Chromatogr.*, **1995**, 18, 649–671.

---

**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu$ m Supelcosil LC-DP (A) or 250  $\times$  4 5  $\mu$ m LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6  
**Injection volume:** 25  
**Detector:** UV 229

---

**CHROMATOGRAM**

**Retention time:** 5.41 (A), 3.18 (B)

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**OTHER SUBSTANCES**

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazinol, mefenamic acid, meperidine, mepherytoin, mepivacaine, mesoridazine, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimizide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotolol, spironolactone, sulfapyrazole, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trifluorpromazine, trimetoprim, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

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**KEY WORDS**

details of plasma extraction

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**REFERENCE**

Koves, E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J. Chromatogr. A*, 1995, 692, 103–119.

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**SAMPLE**

**Matrix:** urine

**Sample preparation:** Condition a Bond Elut C18 SPE cartridge with 3 volumes of MeOH and 2 volumes of water, dry under vacuum. 500  $\mu$ L Urine + 5  $\mu$ g terbutaline, add to the SPE cartridge, wash with 5 volumes of water, elute with 200  $\mu$ L MeOH:50 mM pH 6 potassium phosphate buffer 50:50, add 50  $\mu$ L 50 mM  $\text{Na}_3\text{PO}_4$  to the eluate, pass argon through the mixture, inject a 25  $\mu$ L aliquot.

---

**HPLC VARIABLES**

**Column:** 300 mm long  $\mu$ Bondapak phenyl

**Mobile phase:** MeCN:50 mM pH 5 phosphate buffer 6:94

**Flow rate:** 2.8

**Injection volume:** 25

**Detector:** F ex 280 nm 310

---

**CHROMATOGRAM**

**Retention time:** 2.7

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**Internal standard:** terbutaline (4.1)

**Limit of quantitation:** 500 ng/mL

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**KEY WORDS**

SPE; protect from light; pharmacokinetics

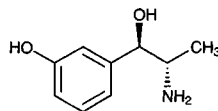
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**REFERENCE**

MacGregor,T.R.; Nastasi,L.; Farina,P.R.; Keirns,J.J. Isolation and characterization of metaproterenol-3-O-sulfate: a conjugate of metaproterenol in human urine, *Drug Metab.Dispos.*, **1983**, *11*, 568–573.

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# Metaraminol



**Molecular formula:**  $C_9H_{13}NO_2$

**Molecular weight:** 167.21

**CAS Registry No.:** 54-49-9, 33402-03-8 (bitartrate)

**Merck Index:** 5993

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**SAMPLE**

**Matrix:** bulk

**Sample preparation:** Dissolve 950 mg metaraminol bitartrate in 50 mL water. Remove a 2 mL aliquot and add it to 5 mL 100  $\mu$ g/mL butylparaben in water, make up to 100 mL with water, inject a 20  $\mu$ L aliquot.

---

**HPLC VARIABLES**

**Column:** 300  $\times$  4 10  $\mu$ m  $\mu$ Bondapak C18

**Mobile phase:** MeOH:water:acetic acid 60:40:1 containing 2.2 g/L dioctyl sodium sulfosuccinate (Dissolve dioctyl sodium sulfosuccinate in MeOH then add water and acetic acid.)

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 254

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**CHROMATOGRAM**

**Retention time:** 11.4

**Internal standard:** butylparaben (8.2)

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**OTHER SUBSTANCES**

**Simultaneous:** methylparaben, propylparaben

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**REFERENCE**

Martin,C.J.; Saxena,S.J. High-performance liquid chromatographic determination of metaraminol bitartrate in the presence of parabens, *J.Pharm.Sci.*, **1980**, *69*, 1459–1461.

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**SAMPLE**

**Matrix:** formulations

**Sample preparation:** Tablets. Dissolve powdered tablets in 10 mM HCl, filter if necessary, inject an aliquot. Injections, solutions. Dilute with 10 mM HCl, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 5  $\mu$ m Partisil-5 ODS-3

**Mobile phase:** MeOH:buffer 30:70 (Buffer was 10 mM sodium 1-octanesulfonate in 0.2% acetic acid.)

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 220

---

**CHROMATOGRAM**

**Retention time:** 22

**Limit of detection:** 50 ng

## OTHER SUBSTANCES

**Simultaneous:** norepinephrine, epinephrine, levonordefrin, isoproterenol, phenylephrine, imputities

## KEY WORDS

tablets; injections; ophthalmic solutions; inhalation solutions

## REFERENCE

Smela, M.J., Jr.; Stromberg, R. Liquid chromatographic determination of six sympathomimetic drugs in dosage forms, *J. Assoc. Off. Anal. Chem.*, **1991**, *74*, 289–291.

## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a 10 µg/mL solution in MeOH, inject a 20 µL aliquot.

## HPLC VARIABLES

**Column:** 125 × 4.9 Spherisorb S5W silica

**Mobile phase:** MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

**Flow rate:** 2

**Injection volume:** 20

**Detector:** E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

## CHROMATOGRAM

**Retention time:** 1.7

## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzocamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotamine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclozine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipiprone, diprenorphine, dipyrindamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, flupromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserine, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamlamine, meclorphenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscipine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxymethamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, pimindine, pimozone, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, pir tramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolantane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenylamine, theophylline, thiethylperazine, thiopropazate, thiopropazine, thioridazine, thiothixene, thonzylamine, timolol, tocinide, tolpropamine, tolycaine, tranlylcypromine, trazodone, trifluoperazine, trifluoperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripeleminamine, triprolidine, tryptamine, verapamil, xylometazoline

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**REFERENCE**

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

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**SAMPLE**

**Matrix:** solutions

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**HPLC VARIABLES**

**Column:** 250 × 4.6 5 µm Partisil ODS-3

**Mobile phase:** MeOH:buffer 30:70 (Buffer was 10 mM octanesulfonic acid in 0.2% acetic acid.)

**Flow rate:** 1

**Detector:** UV 220

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**CHROMATOGRAM**

**Retention time:** 21.5

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**OTHER SUBSTANCES**

**Simultaneous:** epinephrine, isoproterenol, levonordefrin, phenylephrine

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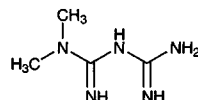
**REFERENCE**

*Phenomenex Catalog*, **1994**, p. 1.077.

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# Metformin



**Molecular formula:** C<sub>4</sub>H<sub>11</sub>N<sub>5</sub>

**Molecular weight:** 129.17

**CAS Registry No.:** 657-24-9

**Merck Index:** 6001

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Add 10 µL 60% perchloric acid to 250 µL plasma. Vortex for 1 min, centrifuge at 12800 g for 3 min. Inject a 50 µL aliquot of the supernatant.

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**HPLC VARIABLES**

**Guard column:** 5 µm Spherisorb CN

**Column:** 250 × 4.6 5 µm Supelcosil LC-CN

**Mobile phase:** MeCN:buffer 40:60 (Buffer was 10 mM KH<sub>2</sub>PO<sub>4</sub> adjusted to pH 3.5 with glacial acetic acid.)

**Flow rate:** 1

**Injection volume:** 50

**Detector:** UV 234

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**CHROMATOGRAM**

**Retention time:** 5.94

**Limit of detection:** 15 ng/mL

**Limit of quantitation:** 60 ng/mL

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**KEY WORDS**

plasma; pharmacokinetics

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**REFERENCE**

Yuen,K.H.; Peh,K.K. Simple high-performance liquid chromatographic method for the determination of metformin in human plasma, *J.Chromatogr.B*, **1998**, *710*, 243–246.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 50  $\mu$ L Plasma + 50  $\mu$ L 100 g/L trichloroacetic acid in water, mix, centrifuge, inject a 20-50  $\mu$ L aliquot of the supernatant

---

**HPLC VARIABLES**

**Column:** 250  $\times$  4.6 10  $\mu$ m Whatman SCX

**Mobile phase:** 100 mM pH 4.42 (NH<sub>4</sub>)H<sub>2</sub>PO<sub>4</sub>

**Flow rate:** 3

**Injection volume:** 20-50

**Detector:** UV 232

---

**CHROMATOGRAM**

**Retention time:** 5

**Limit of detection:** 20 ng/mL

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**OTHER SUBSTANCES**

**Noninterfering:** glibornuride, gliclazide, glipizide, glyburide (glibenclamide)

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**KEY WORDS**

plasma

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**REFERENCE**

Lacroix,C.; Danger,P.; Wojciechowski,F. Microdosage de la metformine plasmatique et intra-érythrocytaire par chromatographie en phase liquide [Microassay of plasma and erythrocyte metformin by high performance liquid chromatography], *Ann.Biol.Clin.(Paris)*, **1991**, 49, 98-101.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** Condition an Amprep 100 mg C8 SPE cartridge (Amersham) with 2 mL MeOH and 1 mL water, do not allow to run dry. 500  $\mu$ L Plasma + 1  $\mu$ g phenformin, add to the SPE cartridge, wash with 2 mL diethyl ether, elute with 500  $\mu$ L MeCN:10 mM pH 3.5 KH<sub>2</sub>PO<sub>4</sub> 70:30. Centrifuge for 5 min or filter (Millipore HV) the eluate, inject an aliquot.

---

**HPLC VARIABLES**

**Guard column:** Guard-pak C18 (Waters)

**Column:** 300  $\times$  3.9 10  $\mu$ m  $\mu$ Bondapak phenyl

**Mobile phase:** MeCN:10 mM KH<sub>2</sub>PO<sub>4</sub> 40:60 adjusted to pH 7 with diethylamine

**Flow rate:** 1.35

**Injection volume:** 50

**Detector:** UV 236

---

**CHROMATOGRAM**

**Retention time:** 2.8

**Internal standard:** phenformin (5.6)

**Limit of detection:** 50 ng/mL

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**KEY WORDS**

plasma; SPE; pharmacokinetics; human; rat

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**REFERENCE**

Huupponen,R.; Ojala-Karlsson,P.; Rouru,J.; Koulu,M. Determination of metformin in plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1992**, 583, 270-273.

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**SAMPLE**

**Matrix:** blood

**Sample preparation:** 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100  $\mu$ L mobile phase, centrifuge at 2800 g for 5 min, inject a 50  $\mu$ L aliquot of



the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

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### HPLC VARIABLES

**Column:** 300 × 3.9 4 μm NovaPack C18

**Mobile phase:** MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic))  $\text{KH}_2\text{PO}_4$  adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

**Column temperature:** 30

**Flow rate:** 0.8

**Injection volume:** 50

**Detector:** UV 231

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### CHROMATOGRAM

**Retention time:** 3.41

**Limit of detection:** <120 ng/mL

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### KEY WORDS

whole blood; plasma; interferences may occur—compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylcegonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; ace-nocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluoperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenopropfen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozone; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tiocloamarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpi-pramine; thioridazine; fenfiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

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### REFERENCE

Tracqui, A.; Kintz, P.; Mangin, P. Systematic toxicological analysis using HPLC/DAD, *J. Forensic Sci.*, **1995**, *40*, 254–262.

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### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Plasma. 500 μL Plasma + 200 μL 30 μg/mL 1-propylbiguanide sulfate in 1.2 mM trichloroacetic acid, vortex for 5 s, let stand for 10 min, centrifuge at 5000 g for 5 min, inject a 100 μL aliquot of the supernatant. Urine. Dilute urine with water, if necessary. 500

$\mu\text{L}$  Urine + 200  $\mu\text{L}$  30  $\mu\text{g/mL}$  1-propylbiguanide sulfate in water, vortex for 5 s, let stand for 10 min, centrifuge at 5000 g for 5 min, inject a 100  $\mu\text{L}$  aliquot of the supernatant.

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#### HPLC VARIABLES

**Column:** 250  $\times$  4.6 Partisil-10 SCX

**Mobile phase:** 30 mM  $(\text{NH}_4)_2\text{H}_2\text{PO}_4$  adjusted to pH 2.4 with orthophosphoric acid

**Column temperature:** 50

**Flow rate:** 3

**Injection volume:** 100

**Detector:** UV 230

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#### CHROMATOGRAM

**Retention time:** 8

**Internal standard:** 1-propylbiguanide sulfate (Synthesis of 1-propylbiguanide is as follows. Heat 9 g propylamine, 7.5 g cyanoguanidine, 11.6 g copper sulfate pentahydrate, 75 mL water in a sealed tube at 100° for 12 h, cool, dilute with 350 mL water, heat to 80°, pass a stream of hydrogen sulfide (Caution! Highly toxic!) through the solution to precipitate copper salts, filter. Evaporate the filtrate under reduced pressure at 100°. Recrystallize the product from hot EtOH and dry it at 100°, mp 194-6°.) (10)

**Limit of detection:** 50-100 ng/mL

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#### OTHER SUBSTANCES

**Noninterfering:** acetaminophen, albuterol, allopurinol, amiloride, amitriptyline, ampicillin, amobarbital, aspirin, caffeine, carbamazepine, chlorpropamide, chlorothiazide, clonidine, dantrolene, dextropropoxyphene, diazepam, digoxin, dioctyl sodium sulfosuccinate, doxepin, ephedrine, ethylestranol, furosemide, glyburide (glibenclamide), hydrochlorothiazide, insulin, isosorbide dinitrate, methyclothiazide, methyl dopa, metoprolol, nitrazepam, nitroglycerin, nortriptyline, oxazepam, phenylbutazone, phenytoin, pindolol, prazosin, prochlorperazine, propoxyphene, propranolol, quinine, sodium chromoglycate, sulfamethoxazole, theophylline, thyroxine, tolbutamide, trimethoprim, verapamil, vitamin B, warfarin

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#### KEY WORDS

plasma

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#### REFERENCE

Charles, B.G.; Jacobsen, N.W.; Ravenscroft, P.J. Rapid liquid-chromatographic determination of metformin in plasma and urine, *Clin. Chem.*, **1981**, 27, 434-436.

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#### SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Plasma. 1 mL Plasma + 250  $\mu\text{L}$  water + 1 mL bromothymol blue solution + 50  $\mu\text{L}$  2  $\mu\text{g/mL}$  propyl biguanide in water, vortex briefly, adjust pH to 7.6-7.8 with a few drops of 0.1% orthophosphoric acid, add 5 mL diethyl ether:dichloromethane 2:1, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove 4 mL of the upper organic phase and add it to 100  $\mu\text{L}$  0.1% tetrabutylammonium hydroxide adjusted to pH 7 with 0.1% orthophosphoric acid, vortex for 1 min, centrifuge at 2000 g for 5 min. Discard the organic phase, heat the aqueous phase at 70-90° for 10 min, inject a 25  $\mu\text{L}$  aliquot of the aqueous phase. Urine. 20  $\mu\text{L}$  Urine + 2 mL bromothymol blue solution + 100  $\mu\text{L}$  20  $\mu\text{g/mL}$  propyl biguanide in water, vortex briefly, adjust pH to 7.6-7.8 with a few drops of 0.1% orthophosphoric acid, add 5 mL diethyl ether:dichloromethane 2:1, vortex for 1 min, centrifuge at 2000 g for 5 min. Remove 4 mL of the upper organic phase and add it to 100  $\mu\text{L}$  1% tetrabutylammonium hydroxide adjusted to pH 7 with 0.1% orthophosphoric acid, vortex for 1 min, centrifuge at 2000 g for 5 min. Discard the organic phase, heat the aqueous phase at 70-90° for 10 min, inject a 25  $\mu\text{L}$  aliquot of the aqueous phase. (Bromothymol blue solution was 1.48 g bromothymol blue in 20 mL 200 mM NaOH, make up to 200 mL with water, let stand at room temperature with occasional mixing for 2 days before use.)

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#### HPLC VARIABLES

**Column:** 150  $\times$  4.6 5  $\mu\text{m}$  Spherisorb ODS 2

**Mobile phase:** MeCN:buffer 8:92 adjusted to pH 4.0 with orthophosphoric acid (Buffer was 50 mM  $\text{K}_2\text{HPO}_4$  containing 3 mM heptanesulfonic acid.)

**Flow rate:** 1

**Injection volume:** 25

**Detector:** UV 234

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**CHROMATOGRAM**

**Retention time:** 3.3

**Internal standard:** propyl biguanide (Synthesis of 1-propylbiguanide is as follows. Heat 9 g propylamine, 7.5 g cyanoguanidine, 11.6 g copper sulfate pentahydrate, 75 mL water in a sealed tube at 100° for 12 h, cool, dilute with 350 mL water, heat to 80°, pass a stream, of hydrogen sulfide (Caution! Highly toxic!) through the solution to precipitate copper salts, filter. Evaporate the filtrate under reduced pressure at 100°. Recrystallize the product from hot EtOH and dry it at 100°, mp 194-6° (Clin.Chem. 1981, 27, 434.) (7.80)

**Limit of detection:** 10 ng/mL

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**KEY WORDS**

plasma

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**REFERENCE**

Keal,J.; Somogyi,A. Rapid and sensitive high-performance liquid chromatographic assay for metformin in plasma and urine using ion-pair extraction techniques, *J.Chromatogr.*, **1986**, 378, 503-508.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Blood. Add plasma or whole blood to MeCN containing propylbiguanide, wash the supernatant with dichloromethane, inject an aliquot. Urine. Mix urine with MeCN containing propylbiguanide, inject an aliquot.

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**HPLC VARIABLES**

**Column:** 250 × 3.6 5 µm Si

**Mobile phase:** MeCN:water 20:80 containing 10 mM (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, pH adjusted to 7.5 with phosphoric acid

**Detector:** UV 235

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**CHROMATOGRAM**

**Internal standard:** propylbiguanide

**Limit of detection:** 4 µg/mL (urine), 10 ng/mL (plasma, whole blood)

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**KEY WORDS**

plasma; whole blood; pharmacokinetics

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**REFERENCE**

Sambol,N.C.; Chiang,J.; Lin,T.; Goodman,A.M.; Liu,C.Y.; Benet,L.Z.; Cogan,M.G. Kidney function and age are both predictors of pharmacokinetics of metformin, *J.Clin.Pharmacol.*, **1995**, 35, 1094-1102.

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**SAMPLE**

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

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**HPLC VARIABLES**

**Guard column:** 20 mm long Symmetry C18

**Column:** 250 × 4.6 5 µm Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 233.4

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## CHROMATOGRAM

**Retention time:** 2.803

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## KEY WORDS

whole blood

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## REFERENCE

Gaillard, Y.; Pépin, G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J. Chromatogr. A*, **1997**, 763, 149-163.

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## SAMPLE

**Matrix:** solutions

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## HPLC VARIABLES

**Column:** 250 × 4.6 5 µm Supelcosil LC-DP (A) or 250 × 4.6 5 µm LiChrospher 100 RP-8 (B)

**Mobile phase:** MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)

**Flow rate:** 0.6

**Injection volume:** 25

**Detector:** UV 229

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## CHROMATOGRAM

**Retention time:** 5.68 (A), 3.25 (B)

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## OTHER SUBSTANCES

**Also analyzed:** acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordi-azepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyrindamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazin-  
dol, mefenamic acid, meperidine, mephénytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyl-  
dopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxe-  
tine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimo-  
zide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, pro-  
pantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocin-  
amide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trifluopro-  
mazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yo-  
himbine, zopiclone

## KEY WORDS

details of plasma extraction

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## REFERENCE

Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, 692, 103–119.

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## SAMPLE

**Matrix:** urine

**Sample preparation:** Mix 2 mL urine with 1 mL 20% NaOH and saturate the mixture with NaCl, add 1 mL MeCN, add 10 mg p-nitrobenzoyl chloride, let stand at room temperature for 1 h, add 10 mg p-nitrobenzoyl chloride, let stand for 1 h, inject a 10  $\mu$ L aliquot of the upper MeCN phase.

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## HPLC VARIABLES

**Column:** 914  $\times$  2.2 37-50  $\mu$ m Bondapak phenyl/Corasil

**Mobile phase:** MeOH:water 40:60

**Flow rate:** 1

**Injection volume:** 10

**Detector:** UV 280

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## CHROMATOGRAM

**Retention time:** 6

**Limit of detection:** 200 ng/mL (using more urine and less MeCN)

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## KEY WORDS

derivatization

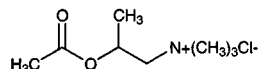
---

## REFERENCE

Ross,M.S.F. Determination of metformin in biological fluids by derivatization followed by high-performance liquid chromatography, *J.Chromatogr.*, **1977**, 133, 408–411.

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# Methacholine chloride



**Molecular formula:** C<sub>8</sub>H<sub>18</sub>ClNO<sub>2</sub>

**Molecular weight:** 195.69

**CAS Registry No.:** 62-51-1

**Merck Index:** 6003

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## SAMPLE

**Matrix:** solutions

**Sample preparation:** Prepare a solution in saline, inject a 20  $\mu$ L aliquot.

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## HPLC VARIABLES

**Column:** 300  $\times$  3.9  $\mu$ Bondapak C18

**Mobile phase:** MeOH:buffer 25:75 (Buffer was 20 mL Low-UV PIC B-7 (Waters) diluted with 480 mL water (10 mM 1-heptanesulfonic acid).)

**Flow rate:** 1

**Injection volume:** 20

**Detector:** UV 210

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## CHROMATOGRAM

**Retention time:** 9.6

**Limit of detection:** 80  $\mu$ g/mL

## REFERENCE

Woodman,T.F.; Johnson,B.; Marwaha,R.K. Determination of methacholine chloride by ion-pair high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1982**, 5, 1341-1348.

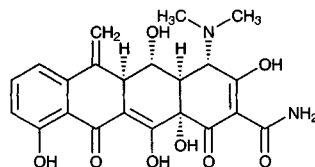
# Methacycline

**Molecular formula:**  $C_{22}H_{22}N_2O_8$

**Molecular weight:** 442.43

**Merck Index:** 6007

**Lednicer No.:** 2 227



## SAMPLE

**Matrix:** blood, urine

**Sample preparation:** Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50  $\mu$ L MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood)  $\mu$ L aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

## HPLC VARIABLES

**Guard column:** 20 mm long Symmetry C18

**Column:** 250  $\times$  4.6 5  $\mu$ m Symmetry C8 (Waters)

**Mobile phase:** Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

**Column temperature:** 30

**Flow rate:** 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

**Injection volume:** 10-30

**Detector:** UV 242.9

## CHROMATOGRAM

**Retention time:** 11.493

## KEY WORDS

whole blood

## REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, 763, 149-163.

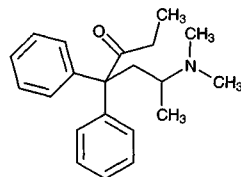
# Methadone

**Molecular formula:**  $C_{21}H_{27}NO$

**Molecular weight:** 309.45

**CAS Registry No.:** 76-99-3, 1095-90-5 (HCl)

**Merck Index:** 6008



## SAMPLE

**Matrix:** blood